Journal of the American College of Cardiology
© 1999 by the American College of Cardiology
Published by Elsevier Science Inc.

Vol. 33, No. 2, 1999
ISSN 0735-1097/99/$20.00
PII S0735-1097(98)00601-9

REVIEW ARTICLE

A Perspective on the Potential Problems With Aspirin as an Antithrombotic Agent: A Comparison of Studies in an Animal Model With Clinical Trials

John D. Folts, PHD, FACC, Andrew I. Schafer, MD,* Joseph Loscalzo, MD, PHD, FACC,†
James T. Willerson, MD, FACC,‡ James E. Muller, MD, FACC§
Madison, Wisconsin; Houston, Texas; Boston, Massachusetts; and Lexington, Kentucky

Aspirin is the most widely prescribed agent to reduce the platelet-mediated contributions to atherosclerosis, coronary thrombosis and restenosis after angioplasty. While aspirin treatment has led to significant reductions in morbidity and mortality in many clinical trials, there are several scenarios in which aspirin may fail to provide a full antithrombotic benefit. The cyclic flow model of experimental coronary thrombosis suggests that elevations of plasma catecholamines, high shear forces acting on the platelets in the stenosed lumen and the presence of multiple, input stimuli can activate platelets through different mechanisms that may lead to thrombosis despite aspirin therapy.

Aspirin therapy is limited because it only blocks some of the input stimuli, leaving aspirin-independent pathways through which coronary thrombosis can be precipitated. These include thrombin and thrombogenic arterial wall substrates such as tissue factor. New agents that block the adenosine diphosphate (ADP) receptor, or regulate platelet free cytosolic calcium, such as direct nitric oxide donors, may be more potent overall than aspirin. Agents that block the platelet integrin GPIIb-IIIa receptor inhibit the binding of fibrinogen to platelets regardless of which input stimuli activate the platelet and, thus, as demonstrated in the cyclic flow model, would be much more potent than aspirin as an antithrombotic agent. The cyclic flow model has been useful in predicting which agents are likely to be of benefit in clinical trials. (J Am Coll Cardiol 1999;33:295–303) © 1999 by the American College of Cardiology

Platelet interactions with damaged arterial walls are known to contribute to the development of thrombosis, atherosclerosis and restenosis after angioplasty, atherectomy or arterial stenting. Ruptured atherosclerotic plaques with platelet-mediated occlusive thrombosis are known to lead to acute coronary syndromes and ischemic stroke. Thus, for over 30 years, there has been an intense search for effective platelet inhibitors.

HISTORICAL BACKGROUND

In the 1950s and 1960s, it was thought that patients with coronary artery disease died from fibrin-mediated "coronary thrombosis;" however, this was widely debated (1), with some investigators suggesting that thrombosis occurred after the fatal myocardial infarction rather than causing it (2,3). Though platelet aggregates were sometimes observed in postmortem thrombi, the specific role of platelets was uncertain at that time.

The studies of Zucker and Weiss et al. in 1968 first showed that aspirin inhibits platelet aggregation in vitro (4,5). Vane demonstrated in 1971 that aspirin blocks the production of prostaglandin synthesis in cells (6), and Smith and Willis observed in the same year that aspirin inhibits platelet aggregation by blocking prostaglandin production in vitro (7,8). In 1971, a trial of daily aspirin was started in 1,239 men who had already had one myocardial infarction, with the hope of preventing a second one. The results reported in 1974 were inconclusive, although they reported a favorable trend (8). The authors concluded that "further trials are urgently required to establish whether or not this aspirin effect is real" (8). Similar trials were begun at the same time.

DEVELOPMENT OF THE CYCLIC FLOW MODEL

In 1972, one of us (J.D.F.) developed a canine model of coronary artery stenosis with intimal damage to mimic a patient with coronary artery disease (PHD thesis, University of Wisconsin, 1972). In this model the circumflex coronary

From the Coronary Thrombosis Research Laboratory, University of Wisconsin Medical School, Madison, Wisconsin; the *Baylor College of Medicine, Medical Service, Houston Veterans Affairs Medical Center, Houston, Texas; the †Department of Medicine, Boston University Medical Center, Boston, Massachusetts; the ‡Department of Internal Medicine, The University of Texas Medical School, Houston, Texas; and the §Division of Cardiology, University of Kentucky Medical Center, Lexington, Kentucky.

Manuscript received December 11, 1997; revised manuscript received September 17, 1998, accepted October 26, 1998.

FEB. 9. 2005 6:30PM PABST PATENT GROUP NO. 3080 P. 58

JACC Vol. 33, No. 2, 1999
February 1999:295–303

Abbreviations and Acronyms

ADP = adenosine diphosphate
AMP = adenosine monophosphate
CFRs = cyclic flow reductions
C7E3 = chimeric monoclonal antibody to the platelet GPIIb-IIIa receptor
EDRF = endothelial-derived relaxing factor
GMP = guanosine monophosphate
NO = nitric oxide
PDE = phosphodiesterase

artery of anesthetized dogs was dissected out and an electromagnetic flowmeter probe was placed on the artery to continuously measure coronary blood flow. A plastic encircling cylinder was then placed around the outside of the coronary artery distal to the flow probe. The cylinder produced a 70% stenosis and intimal damage. The coronary blood flow was shown to periodically decline to zero, producing transient myocardial ischemia. The flow would then suddenly spontaneously return to normal and the ischemia would be resolved. It was subsequently shown that the flow decline was caused by a platelet-mediated thrombus, gradually forming in the narrowed lumen, which cut off the blood flow. When the friable, loosely packed thrombus broke up and embolized distally, the blood flow was suddenly restored. This model thus demonstrated "in vivo" that periodic, acute platelet-mediated thrombotic occlusion, followed by embolization, could occur in stenosed and intimally damaged canine coronary arteries producing cyclic reductions in coronary blood flow. In addition, the thrombus was produced by platelet interaction with damaged arterial walls, and histologically consisted of mainly platelets: some red cells but little fibrin. These cyclic flow reductions (CFRs) that lead to experimental myocardial ischemia, and potentially lethal arrhythmias, were shown to be prevented by aspirin but not by heparin (9). This cyclic flow model has been described as representing some of the events occurring in a patient with unstable angina and useful for studying mechanisms of unstable angina (10–12). The model allows a reproducible pattern of recurrent thrombosis to be established that then permits testing of potential antithrombotic agents (11–15). A key feature of the model is the provision of an internal control for each animal (11–14). The model also permits potential antiplatelet agents to be given intravenously or orally (13). Finally, the model also allows for studies in anesthetized or awake, unsedated animals (12,14,15). Although widely used and quoted, this animal model, however, does not mimic very well some of the conditions existing in patients with atherosclerotic narrowing of coronary or peripheral arteries. The model does not have many of the substrates found in ruptured atherosclerotic plaques, especially the very thrombogenic lipid core (16). In addition, risk factors, such as smoking, diabetes, hyperlipidemia or hypertension, associated with increased platelet activity and vascular disease, are not usually associated with this model (17).

There are not, however, many alternative models that permit repetitive experimental measurements of in vivo platelet activity or platelet interactions with arterial walls over time. Evaluation of ex vivo platelet "function" or level of activity is labor intensive, difficult and usually can be done at only one point in time (18,19). Other animal models (11) or ex vivo perfusion chambers (20) offer some very distinct advantages but possess unique disadvantages as well, which are beyond the scope of this review. The authors are most familiar with the use of the cyclic flow model, which has recently been reviewed (11,21,22).

Cyclic flow reductions were first observed in 1978 in the popliteal artery of patients with peripheral vascular disease having intermittent claudication (23). In addition, cyclic flow variations have now been observed in patients at the time of angioplasty (24,25). Finally, the frequency and severity of cyclic flow alterations and platelet aggregation predicted the severity of neointimal proliferation after experimental stenosis and endothelial injury in conscious dogs (15). The cyclic flow variations in both anesthetized and conscious dogs and humans have been compared with the clinical manifestations of unstable angina and their sequelae by a number of authors (10,13,15,26). Seven antiplatelet drugs, including aspirin, have been evaluated in the cyclic flow model in several animal species, and were found to be effective in vivo as platelet inhibitors (22). These drugs have also been used in clinical trials with encouraging results (22). Two drugs, dipyridamole and prostacyclin, failed to be effective at clinical doses with acceptable hemodynamics in the cyclic flow model, and also were shown to not be effective platelet inhibitors in a variety of clinical trials (22,27).

MECHANISMS OF PLATELET ACTIVATION AND AGGREGATION: INPUT STIMULI

Platelet activation in vivo leading to platelet-mediated coronary thrombosis can be considered to occur in several linked phases (Fig. 1) (17,22). First, platelets adhere to a site of vascular intimal injury where the antithrombotic properties of dysfunctional endothelium are lost. Subsequently, many input stimuli can activate platelets in vivo and ultimately cause adhesion, clumping, aggregation and thrombus formation. These input stimuli or agonists can act independently, and yet some are also synergistic with one another. The net effects of these input stimuli are to raise platelet cytosolic calcium, which triggers contraction of platelet actin and myosin fibrils, leading to platelet shape change, and the release reaction. This is the calcium mobilization phase shown in the center of Figure 1. The final phase is the activation and exposure of the membrane glycoprotein IIb–IIIa fibrinogen receptor, shown on the right of Figure 1. Many studies have been done that address ways to block the various input stimuli and thereby decrease platelet activity.

PAGE 58/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

FEB. 9. 2005 6:31PM PABST PATENT GROUP NO. 3080 P. 59

JACC Vol. 33, No. 2, 1999
February 1999:295–303

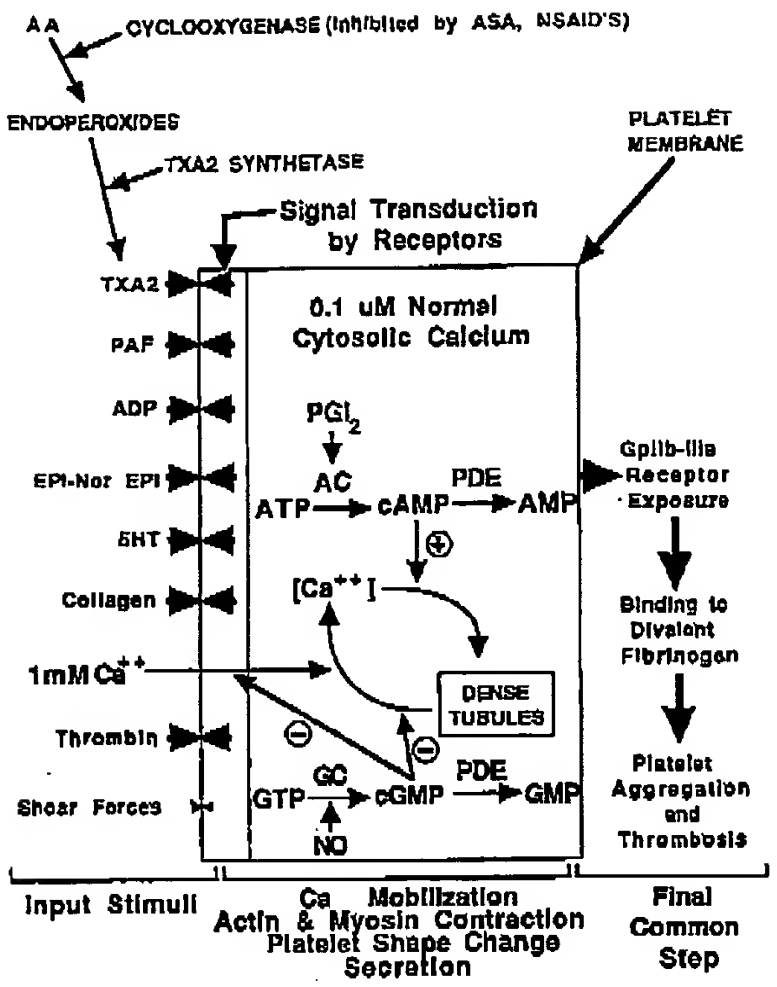

Figure 1. Schematic diagram showing the different input stimuli on the left that can activate platelets in vivo. These stimuli act by signal transduction through receptors to ultimately increase the free cytosolic Ca^{2+} level in platelets. In the center are the calcium mobilization reactions that interact with the receptors and regulate cytosolic free Ca^{2+} ($[Ca^{2+}]i$). Adenosine triphosphate (ATP) is converted to cyclic adenosine monophosphate (cAMP) by the enzyme adenylate cyclase (AC). cAMP is broken down to AMP by the enzyme phosphodiesterase. When cAMP is elevated, for example by prostacyclin (PGI_2) binding to a specific receptor and stimulating adenylate cyclase (AC), some Ca^{2+} is stored in the dense tubules. This reduces cytosolic free Ca^{2+} and decreases the level of platelet activation. Another regulator of free cytosolic Ca^{2+} is cyclic GMP. Guanosine triphosphate (GTP) is converted to cGMP by guanylate cyclase (GC). cGMP is broken down by a phosphodiesterase to produce GMP. When cGMP is elevated by stimulation of GC by NO, free cytosolic Ca^{2+} is reduced by two mechanisms. Ca^{2+} is inhibited from entering the platelet from the plasma, and also Ca^{2+} is inhibited from leaving the dense tubules. This also reduces the available level of cytosolic Ca^{2+}. Thus modulating free cytosolic Ca^{2+} can increase or decrease platelet activity. The final step in platelet-mediated thrombosis is the exposure/activation of the platelet glycoprotein IIb–IIIa fibrinogen receptor, which binds to fibrinogen to create a platelet aggregate.

CFRs in the canine model with coronary artery constriction and intimal injury, and in patients with periodic coronary thrombosis causing unstable angina, are caused by a variety of different mediators or input stimuli singly and in combination, besides thromboxane A_2, which include serotonin, thrombin, ADP, epinephrine, platelet-activating factor, oxygen-derived free radicals as well as shear stress, shown on the left of Figure 1 (22,28–32). In this review, we will attempt to compare effective experimental platelet inhibitors in the CFR model with clinical trials of the same platelet inhibitors.

ASPIRIN AS A PLATELET INHIBITOR

There has been considerable interest and success with the use of aspirin to prevent platelet-mediated thrombotic events in stenosed coronary and cerebral arteries.

The Antiplatelet Trialists' meta-analysis showed a 25% reduction in the incidence of cardiovascular events with the use of aspirin (33). The Physicians' Health Study showed a 44% reduction in the first incidence of myocardial infarction (34). Identification of those patients and those types of events likely to benefit from aspirin treatment and those not likely to do so may serve as a means to detect those patients who may be at greater risk in a clinical trial. For example, the morning increase in myocardial infarction and stroke is accompanied by a catecholamine surge in many but not all patients, producing increased platelet activity (17,35,36). The Physicians' Health Study demonstrated a 59% reduction in morning myocardial infarctions in patients treated with aspirin compared with controls, which is encouraging, although a relatively small number of patients were in this subgroup (37). A similar, although not comparable study, was done with British doctors receiving daily aspirin (500 mg/d), which did not show a significant decrease in a first myocardial infarction (38).

Recently, the benefits of aspirin for primary and secondary prevention of occlusive vascular disease were extensively reviewed (39,40). There is a clear indication for the use of aspirin to reduce the risk of death from cardiovascular causes or nonfatal myocardial infarction and stroke in patients with unstable angina or a history of myocardial infarction, transient cerebral ischemia or stroke (39,40).

These clear benefits of aspirin therapy described in clinical trials of patients with atherothrombotic disease are significant. However, the animal model suggests that there may be some potential problems with aspirin and other platelet inhibitors that should be assessed in clinical trials. Therefore, we will examine some of these problems, compare them with clinical trials and speculate on why some investigators feel that aspirin is not a very potent platelet inhibitor (17,41).

INPUT STIMULI OR AGONISTS THAT INCREASE IN VIVO PLATELET ACTIVITY BUT ARE ONLY PARTIALLY BLOCKED BY ASPIRIN

It has been shown in the animal model that the antithrombotic effect of aspirin can be overcome and periodic platelet thrombus formation restored by the experimental infusion of epinephrine (42), or by ventilating the animals with cigarette smoke, producing free radicals and acute elevations in endogenous catecholamines (43). In another study the antithrombotic effects of aspirin and the prothrombotic

PAGE 59/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

FEB. 9. 2005 6:31PM PABST PATENT GROUP NO. 3080 P. 60

JACC Vol. 33, No. 2, 1999
February 1999:295–303

effects of epinephrine were compared simultaneously by using the cyclic flow model and an ex vivo shunt through a perfusion chamber in the same dogs. The perfusion chamber had human fibrillar collagen as the thrombogenic surface (44). The CFRs and ex vivo thrombosis in the chamber were significantly inhibited by 10 mg/kg of aspirin given intravenously. However, when epinephrine 10 μg/min was infused intravenously for 5 min, both the CFRs were restored in the stenosed arterial lumen and a significant increase in thrombus formation occurred in the perfusion chamber (44). Platelet activation produced in vivo by many of the input stimuli, including thrombin, shown in Figure 1, is enhanced by a synergistic effect with elevations of circulating catecholamines (45). Catecholamine-enhanced thrombogenesis and catecholamine-dependent vasoconstriction may be important in humans because they may be the link between emotional stress such as anger, circadian variation of activity and heavy physical exercise and the onset of acute cardiovascular disease (46). Hypercatecholaminergic states, which enhance thrombosis and vasoconstriction, may trigger an acute coronary syndrome if they coincide with, or help to cause, the rupture of an atherosclerotic plaque (17,46). Thus, the animal model would suggest that we should look at the levels of catecholamines in this subset of patients to see how well any platelet inhibitor protects against acute thrombosis when catecholamines are elevated.

The animal model also demonstrates that high shear forces acting on platelets passing through severely narrowed stenoses can also overcome the inhibitory effects of aspirin (47,48). Furthermore, direct shear stress-induced platelet aggregation is not significantly inhibited by aspirin (17,47,48). Finally, it has been shown recently that epinephrine acts synergistically with shear stress to induce platelet aggregation, and that this synergistic interaction is likewise unaffected by aspirin (49).

CLINICALLY HYPERACTIVE PLATELETS

Some patients with coronary artery disease have more active platelets than healthy control subjects. Patients with diabetes mellitus type I and type II, hypercholesterolemia and some forms of hypertension have increased platelet aggregability (17). In one study the hyperactive platelets of insulin-dependent diabetics were inhibited less by aspirin than the platelets of nondiabetics (50). In addition, studies on the blood of patients who had an atherothrombotic stroke within the previous 72 h show they have hyperreactive platelets that are more susceptible to shear-induced aggregation. This hyperactivity was not significantly decreased by aspirin treatment (51). There are some patients who are resistant to the effects of aspirin and whose platelets are not inhibited significantly by aspirin. In another study of 180 post-stroke patients, 120 showed a good platelet inhibitory response 12 h after 500 mg of aspirin postoperatively (aspirin responders) (52). However, 60 patients did not show a significant platelet inhibitory response 12 h after 500 mg of aspirin PO (secondary aspirin nonresponders). After a 24-month follow-up, where all patients received 3 × 500 mg of aspirin per day, a second fatal or nonfatal stroke or myocardial infarction occurred in 4.4% of the aspirin responders, but these events occurred in 40% of the aspirin nonresponders ($p < 0.001$) (52). Thus, there appear to be at least some individuals identified whose platelets may not be significantly inhibited by daily aspirin therapy, and they go on to have an increased incidence of ischemic vascular events (52). These small but controlled studies should give us insight and clues as to what to look for when planning and interpreting future trials, and also interpreting results obtained with the animal model. The potential clinical importance of these observations was recently demonstrated by Eichhorn et al. in patients with unstable angina undergoing coronary angioplasty (24). CFRs measured with an intravascular doppler flowmeter and thought to be platelet mediated were documented in these patients despite aspirin and heparin treatment (24). The occurrence of CFRs in these aspirin-treated patients may have resulted from the combination of elevated catecholamines and turbulence in the rough, stenosed lumen producing turbulent flow, which increased shear forces acting on the platelets during the procedure, or they may have been aspirin resistant (52). In addition, the ruptured atherosclerotic plaque leading to unstable angina also exposes materials that may provoke thrombosis by mechanisms that aspirin does not block, such as tissue factor (17). Plasma epinephrine levels measured in patients during coronary angiography procedures range from 100 to 300 pg/ml compared with 50 to 75 pg/ml in healthy controls (unpublished observations, J.D.F.). This increase in plasma concentration is enough to overcome the inhibitory effects of aspirin in vivo and in vitro (42). In a more recent study, CFRs were also observed in patients treated with aspirin and heparin at the time of angioplasty. The CFRs were subsequently abolished with an antibody that blocks the platelet GPIIb-IIIa receptor (25). This is a much more potent platelet inhibitor than aspirin (see below).

Patients with atherosclerotic narrowing of arteries and subsequent plaque rupture may be in a worse situation than the animal cyclic flow model used to study platelet interactions with damaged arterial walls. In the presence of experimental acute deep arterial injury, similar to that created by balloon angioplasty or rupture of an atherosclerotic plaque, aspirin reduces thrombus formation by only 55% (53). Exposure of tissue factor deep in the arterial wall will stimulate thrombosis, and this effect is not inhibited by aspirin (17). In addition, fresh mural thrombus is a very potent stimulus for further growth of thrombus in spite of aspirin therapy (17,54). Thrombin is also a major factor in fresh mural thrombus, and aspirin does not block thrombin activation of platelets to any significant degree (17,55,56).

The cyclic flow model demonstrated some inhibition of platelet activation by heparin (30). However, the mecha-

PAGE 60/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

FEB. 9. 2005 6:32PM PABST PATENT GROUP NO. 3080 P. 61

JACC Vol. 33, No. 2, 1999
February 1999:295–303

nism by which heparin inhibits thrombin production is not very effective within the thrombus itself, where the heparin-antithrombin III complex is excluded. There is recent interest in the use of more thrombin-specific inhibitors such as hirudin or hirulog (54). These agents are much more effective at inhibiting the growth of new thrombus on fresh mural thrombus than aspirin (54).

ADP RECEPTOR BLOCKADE

Ticlopidine and its analog, clopidogrel, are thienopyridine derivatives that exert their antiplatelet action by inhibiting adenosine phosphase (ADP) binding to its platelet receptors, thereby blocking ADP-induced platelet aggregation (41). These agents may also partially inhibit platelet response to other stimuli, which act in part by causing the release of ADP from endogenous platelet granule pools.

Based on large randomized trials, ticlopidine was found to be at least as effective as aspirin for the prevention of subsequent vascular attacks and death in patients with transient ischemic attacks, completed atherothrombotic strokes, unstable angina and intermittent claudication (57,58). Since ticlopidine inhibits primarily ADP-induced platelet aggregation, it has been suggested that a lower dose of ticlopidine, combined with aspirin to block thromboxane production, might be more effective (58).

A new thienopyridine derivative, clopidogrel, is currently being studied. The cyclic flow dog model demonstrated that clopidogrel was very effective at inhibiting platelet activity and abolishing cyclic flow reductions when given as an intravenous infusion (32). A randomized blinded trial of clopidogrel versus aspirin in patients at risk of ischemic events, the CAPRIE trial, suggested that clopidogrel is more effective than aspirin (59). This was the first study of an antiplatelet drug to include patients from the clinical subgroups of ischemic cerebrovascular, cardiac and peripheral arterial disease under a common protocol. Clopidogrel has fewer side effects than ticlopidine and appears to be more potent (59). This study suggests that the ADP input stimuli may be more significant than the thromboxane A_2 input stimuli to platelet activation. The pharmacology of clopidogrel has recently been reviewed (60).

Given these multiple pathways or input stimuli for platelet activation and the fact that aspirin primarily inhibits only one pathway, it is interesting to note that aspirin provides as much protection against acute atherothrombotic events as has been demonstrated in numerous clinical trials (17,39,40).

PLATELET CYTOSOLIC CALCIUM MOBILIZATION

Regulation of cyclic AMP and cGMP. Many tissues, including cardiac, skeletal and vascular smooth muscle, have their level of activity regulated in part by the level of cyclic nucleotides, ie, cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP). These cyclic nucleotides regulate the level of cytosolic free $[Ca^{2+}]i$ (22).

The many input stimuli that can potentially activate platelets also act through this common mechanism, ie, to increase the level of free cytosolic calcium in the platelet. Thus, it would seem logical to attempt to decrease platelet function by regulating the cytosolic ionized calcium concentration ($[Ca^{2+}]i$), shown in the center of Figure 1. Two of the primary mechanisms for regulating platelet cytosolic calcium are the levels of cyclic AMP and cyclic GMP. These two general endogenous systems are potential targets for attenuation of platelet activation by sequestering cytosolic calcium. Prostacyclin and its analogues increase cyclic AMP, while endothelial-derived relaxing factor (EDRF) and nitric oxide (NO) analogues increase cyclic GMP. In either case, by increasing cyclic AMP or cyclic GMP, cytosolic Ca^{2+} flux and platelet activation are reduced (61). In Figure 1, the input stimuli acting through specific receptors all have the same basic effect on platelets, which is to raise cytosolic calcium.

NO DONORS TO RAISE CYCLIC GMP

Organic nitrates are modest inhibitors of in vitro platelet aggregation (62). We have shown in the cyclic flow model that intravenous nitrates or NO donors, such as nitroglycerin, (63) and to a greater extent the direct NO donors, sodium nitroprusside (64) or the direct NO donor, NO gas and S-NO-cysteine (65), can effectively inhibit platelet activity in vivo at clinically relevant doses and prevent experimental coronary artery thrombosis. They do this, at least in part, by elevating platelet cGMP (64,65). In addition, these NO donors protect against renewal of thrombus formation and CFRs in the cyclic flow model by elevated plasma epinephrine, which aspirin does not (64,65). Studies in healthy subjects and patients with stable angina pectoris have shown that transdermal glyceryl trinitrate and sublingual nitroglycerin (both NO donors) can inhibit platelet activity ex vivo (66–68). This was shown both by ex vivo whole blood aggregation studies and by drawing patient blood through a perfusion chamber. The patient blood was exposed to porcine aortic media in the chamber at high shear comparable with that created by coronary artery stenosis (67). The platelet aggregation studies showed significant inhibition by nitrates to both collagen and ADP agonists. Aspirin does not completely inhibit ADP or collagen-induced aggregation, but NO does (69). Aspirin also does not effectively inhibit platelet deposition on damaged arterial walls in perfusion chambers (44). Thus, both the cyclic flow model, patient ex vivo platelet aggregation studies and drawing NO-treated human blood over exposed porcine media all showed inhibition of platelet activity by the NO donor.

It has been speculated that some of the beneficial clinical effects of organic nitrates in unstable angina and the onset of myocardial infarction are due to their platelet inhibiting properties (70–72). It is known that during an evolving myocardial infarction (MI), the patient's plasma cat-

PAGE 61/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

FEB. 9. 2005 6:33PM PABST PATENT GROUP NO. 3080 P. 62

JACC Vol. 33, No. 2, 1999
February 1999:295–303

echolamines are elevated. Studies with the animal model would suggest that an NO donor may be of more benefit in this particular situation than aspirin, but this will require a well-designed study to clearly demonstrate this.

In two large clinical trials, ISSIS-4 and GISSI-3, there was no major overall reduction in mortality when glyceryl trinitrate was given for 6 weeks after an acute MI (73,74). This may be due to starting the nitrate too late, ie, 6 weeks after the acute MI, and because nitrates are not as potent as direct NO donors (70).

It may be that the use of better NO donors than nitrates, studied at a time when platelet-vessel wall interaction is more intense, such as during angioplasty or unstable angina, may make it easier to detect the platelet inhibitory effects of NO.

For example, in seven control patients, coronary angioplasty, which produces deep arterial injury, caused an increase in platelet activation as demonstrated by a rise in platelet surface expression of P-selectin and the platelet fibrinogen receptor, the integrin GPIIb-IIIa, 5–10 min after the balloon dilation. This occurred in spite of treatment with aspirin, nitroglycerin and heparin. In six patients, the intracoronary infusion of S-nitrosoglutathione, a much better NO donor than nitroglycerin, started 10 min before angioplasty, significantly inhibited this percutaneous transluminal coronary angioplasty–induced increase in platelet surface expression of P-selectin and the platelet integrin GPIIb-IIIa (75). Thus, the animal model and a group of small preliminary patient studies suggest the NO donors may significantly reduce in vivo thrombosis by elevating platelet cyclic GMP levels. The NO donors raise platelet cyclic GMP levels, which lowers $[Ca^{2+}]i$ levels. This is a biochemical step occurring after the input stimuli, and the animal model studies suggest that NO is likely to prevent renewal of platelet activity by elevated catecholamine levels (Fig. 1). Well-planned clinical trials should help to determine if NO donors can inhibit platelet activity better than aspirin and whether this has clinical relevance.

CALCIUM CHANNEL BLOCKERS

Since many platelet functions are dependent on the level of intracellular calcium for optimal activation, calcium channel blockers were tested in the animal model. Early in vitro and ex vivo studies of the effects of calcium channel blockers showed some inhibition of platelet aggregation but required higher doses than can be achieved in vivo.

We tested the acute effect of several calcium channel blockers, including verapamil (0.4 mg/kg), in the cyclic flow model at doses comparable with those given intravenously clinically, and found they did not significantly decrease in vivo platelet activity (76). We did observe, however, that this low dose of verapamil was synergistic with aspirin, abolished acute thrombus formation and protected against renewal of platelet thrombus formation with elevated plasma epinephrine levels (76). A clinical study using a higher dose of slow release verapamil (240 mg/d) given for 7 d did show that this higher dose of verapamil significantly decreased thrombus formation when patient blood was drawn over damaged porcine aortic media at shear forces typical of an arterial stenosis (77). The verapamil also decreased thrombin induced ex vivo platelet aggregation. In this study, the higher dose of verapamil was quite effective by itself, and there was no evident synergism when aspirin (325 mg/day) was added to the verapamil treatment.

Another group of calcium channel blockers, called dihydropyridines, have been developed. The calcium channel blocker, amlodipine, a newer dihydropyridine, was studied in the cyclic flow animal model, and it was found that 0.4 mg/kg IV did significantly inhibit platelet function. This dose was also synergistic with aspirin (78). The amlodipine alone provided significant protection against the renewal of platelet activity by elevated plasma epinephrine levels, while the addition of 5 mg/kg of aspirin gave complete protection (78). A large overview of trials with calcium channel blockers in acute myocardial infarction and unstable angina published in 1989 showed no apparent reduction in the risk of initial or recurrent infarction or death (79). However, the calcium channel blockers are a very diverse group of compounds and have been prescribed in a range of doses, along with other medications. Thus, it may be that more discrete studies with clearer endpoints are needed, to show if newer calcium channel blockers, like amlodipine, can inhibit acute thrombotic events.

PHOSPHODIESTERASE INHIBITORS

Another approach to reduce platelet cytosolic calcium is by raising cyclic AMP with the use of a phosphodiesterase (PDE) inhibitor, shown in the center of Figure 1. Dipyridamole was thought to inhibit platelet activity in part because it is a PDE inhibitor, but it appears to be more effective on vascular smooth muscle PDEs than platelet PDEs (80). Dipyridamole was totally ineffective at inhibiting platelet activity and acute thrombus formation in the cyclic flow model, added nothing to the effects of aspirin (81) and was of very limited value in most clinical trials (27).

FINAL COMMON PATHWAY: PLATELET GPIIB/IIIA INTERACTION WITH FIBRINOGEN

The final step in platelet activation, regardless of the type of input stimuli involved, is the exposure and activation of the platelet fibrinogen receptor, the integrin GPIIb-IIIa (Fig. 1). In the cyclic flow animal model of unstable angina, a monoclonal antibody (7E3), now called abciximab or ReoPro™, to this receptor was a very potent platelet inhibitor and completely protected against periodic acute platelet-mediated thrombus formation and CFRs. The CFRs were not renewed with elevations in plasma epinephrine combined with severe increases in the amount of stenosis and

PAGE 62/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

FEB. 9. 2005 6:33PM PABST PATENT GROUP NO. 3080 P. 63

shear forces (82). However, there was prolongation of the template bleeding time. Dr. Willerson's group showed that some patients with acute unstable angina at the time of angioplasty, treated with aspirin, still had CFRs measured with a Doppler catheter (24). In another patient group with unstable angina, these authors showed that in those patients who had CFRs in spite of aspirin treatment, the infusion of the 7E3 antibody abolished the CFRs (25). A chimeric version (C7E3) of this antibody was produced to decrease the potential antigenicity. Adding this agent to aspirin and heparin produced a significant decrease in the acute thrombotic complications of coronary artery angioplasty compared with aspirin and heparin alone (83), and the benefits persisted for at least 6 months (84). In addition, C7E3 added to aspirin and heparin therapy significantly reduced thrombotic complications and myocardial infarction during angioplasty in patients with refractory unstable angina (85). Angioplasty produces deep arterial injury exposing tissue factor and the thrombogenic materials beneath the fibrous cap overlying atherosclerotic lesions. Thus, it is not surprising that C7E3 combined with aspirin and heparin was much more potent that aspirin and heparin alone.

As suggested in Figure 1, blocking the glycoprotein IIb-IIIa fibrinogen receptor, ie, the final common pathway, is likely to be the most potent way to inhibit platelet activity in vivo. However, with this great potency comes increased risk of bleeding. A group of glycoprotein IIb-IIIa receptor inhibitors is now available for both parental and oral use. Many of these are being used in clinical trials and they also appear to be much more effective than aspirin for unstable angina and coronary angioplasty. These data have recently been extensively reviewed (86,87).

SUMMARY

It would appear that with the exception of more specific thrombin inhibitors, and possibly blockers of the ADP receptor, there is little advantage in attempting to individually block the many input stimuli that activate platelets as a means to inhibit platelet activity and provide a better antithrombotic effect. Consideration of the primary phases of platelet activation and the place where a platelet inhibitor acts, as illustrated in Figure 1, may facilitate an understanding of the efficacy and potency of platelet-inhibiting drugs as they become available. Aspirin will continue to be widely used for patients with vascular disease (5,6); however, there are a number of situations in which increased thrombotic risk requires the use of a more potent platelet inhibitor than aspirin. Conditions such as unstable angina, angioplasty, coronary stenting and thrombolysis are likely to require more potent platelet inhibitors. In these acute clinical situations, the fibrous cap over an atherosclerotic plaque has been ruptured or may be ruptured by interventional procedures. This produces deep arterial injury and exposes a much more thrombogenic surface.

The use of more potent NO donors may offer an alternative means of reducing platelet activity by elevating platelet cyclic GMP levels and lowering platelet cytosolic calcium. An attractive feature of this form of intravenous therapy is that platelet function will return to normal when the use of the NO donor is terminated. In those instances when a very potent platelet inhibitor is needed, the glycoprotein IIb-IIIa fibrinogen receptor antagonists can be utilized. As oral forms of GPIIb-IIIa receptor antagonists become available, they may be useful for reducing the problems of restenosis and intimal hyperplasia that occur after angioplasty, atherectomy and arterial stenting, which are thought to be due in part to significantly increased platelet interactions with severely damaged arterial walls, and for which aspirin has not been very effective.

Reprint requests and correspondence: Dr. John D. Folts, Director, Coronary Thrombosis Research Laboratory, Professor of Medicine, Cardiology, University of Wisconsin Medical School, 600 Highland Avenue, H6/379, Madison, Wisconsin 53792-3248. E-mail: JdF@medicine.wisc.edu.

REFERENCES

1. Branwood AW, Montgomery GL. Observations on the morbid anatomy of coronary artery disease. Scot Med J 1956;1: 367–70.
2. Erhlich JC, Shinohara Y. Low incidence of coronary thrombosis in myocardial infarction. A restudy by serial block technique. Arch Pathol 1964;78:432–7.
3. Roberts WC, Buja LM. The frequency and significance of coronary arterial thrombi and other observations in fatal acute myocardial infarction. A study of 107 necropsy patients. Am J Med 1972;52;425–43.
4. Zucker MB, Peterson J. Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicylic acid ingestion. Proc Soc Exp Biol Med 1968; 127:547–51.
5. Weiss HJ, Aledort LM. Impaired platelet/connective-tissue reaction in man after aspirin ingestion. Lancet 1967;2:495–7.
6. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature [New Biol] 1971;231: 232–5.
7. Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nature [New Biol] 1971; 231:235–7.
8. Elwood PC, Cochrane AL, Burr ML, et al. A randomized controlled trial of acetyl salicylic acid in the secondary prevention of mortality from myocardial infarction. Br Med J 1974;1:436–40.
9. Folts JD, Rowe GG. Platelet aggregation in partially obstructed vessels and their elimination with aspirin. Circulation 1976;54:365–70.
10. Sherry S. Aspirin and antiplatelet drugs: the clinical approach. CVR & R 1984;5:1208–19.
11. Bush LR, Shebuski RJ. In vivo models of arterial thrombosis and thrombolysis. FASEB J 1990;4:3087–98.
12. Ikeda H, Koga Y, Kuwano K, et al. Cyclic flow variations in a conscious dog model of coronary artery stenoses and endothelial injury correlate with acute ischemic heart disease syndromes in humans. J Am Coll Cardiol 1993;21:1008–17.
13. Demrow HS, Slane PR, Folts JD. Administration of wine and

PAGE 63/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

FEB. 9. 2005 6:34PM PABST PATENT GROUP NO. 3080 P. 64

JACC Vol. 33, No. 2, 1999
February 1999:295-303

grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. Circulation 1995;91: 1182–8.
14. Folts JD, Gallagher KP, Rowe GG. Cyclical flow reductions in arterial blood flow in stenosed canine coronary arteries: Vasospasm or platelet aggregation. Circulation 1982;65:248–55.
15. Willerson JT, Yao SK, McNatt J, et al. Frequency and severity of cyclic flow alterations and platelet aggregation predict the severity of neointimal proliferation following experimental coronary stenosis and endothelial injury. Proc Natl Acad Sci USA 1991;88:10624–8.
16. Fernández-Ortiz A, Badimon JJ, Falk E, et al. Characterization of the relative thrombogenicity of atherosclerotic plaque components: Implications for Consequences of plaque rupture. J Am Coll Cardiol 1994;23:1562–9.
17. Fuster V. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 1994;90: 2126–46.
18. George JN, Shattil SJ. The clinical importance of acquired abnormalities of platelet function. N Engl J Med 1991;324: 27–39.
19. Tiffany ML. Technical considerations for platelet aggregation and related problems. CRC Crit Rev Clin Lab Sci 1983;19: 27–69.
20. Sakariassen KS, Joss R, Muggli R, et al. Collagen type III induced ex vivo thrombogenesis in humans: Role of platelets and leukocytes in deposition of fibrin. Arteriosclerosis 1990; 10:276–84.
21. Folts J. An in vivo model of experimental arterial stenosis, intimal damage, and periodic thrombosis. Circulation 1991; 83(suppl IV):3–14.
22. Folts JD. Drugs for the prevention of coronary thrombosis: from an animal model to clinical trials. J Cardiovasc Drugs Therapy 1995;9:31–43.
23. Folts JD, Detmer D, Nadler R. Possible platelet thrombi formation in dogs and human femoral arteries. Texas Heart Institute Journal March 1982;9:19–27.
24. Eichhorn EJ, Grayburn PA, Willard JE, et al. Spontaneous alterations in coronary blood flow velocity before and after coronary angioplasty in patients with severe angina. J Am Coll Cardiol 1991;17:43–52.
25. Anderson HV, Kirkeeide RL, Krishnaswami A, et al. Cyclic Flow variations after coronary angioplasty in humans: Clinical and angiographic characteristics and elimination with 7e3 monoclonal antiplatelet antibody. J Am Coll Cardiol 1994;23: 1031–7.
26. Willerson JT, Campbell WB, Winniford MD, et al. Conversion from chronic to acute coronary artery disease: speculation regarding mechanisms. Am J Cardiol 1984;54:1349–54.
27. Fitzgerald GA. Dipyridamole. N Engl J Med 1987;316:1247–57.
28. Willerson JT, Golino P, Eidt J, Campbell WB, Buja LM. Specific platelet mediators and unstable coronary artery lesions. Experimental evidence and potential clinical implications. Circulation 1989;80:198–205.
29. Ashton JH, Buja LM, Campbell WB, et al. Serotonin and thromboxane A_2/prostaglandin H_2 receptor activation cooperatively mediate cyclic flow variations in dogs with severe coronary artery stenoses. Circulation 1987;76:952–9.
30. Eidt JF, Allison P, Buja LM, et al. Thrombin is an important mediator of platelet aggregation in stenosed and endothelially-injured canine coronary arteries. J Clin Invest 1989;84:18–27.
31. Torr A, Noble MIM, Folts JD. Inhibition of acute platelet thrombosis in stenosed canine coronary arteries by the specific serotonin S_2 receptor antagonist ritanserin. Cardiovasc Res 1990;26:465–70.
32. Yao SK, Ober JC, McNatt J, et al. ADP plays an important role in mediating platelet aggregation and cyclic flow variations in vivo in stenosed and endothelium-injured canine coronary arteries. Circ Res 1992;70:39–48.
33. Antiplatelet Trialists' Collaboration. Collaborative overview of randomized trials of antiplatelet therapy. I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Br Med J 1994;308:81–106.
34. Steering Committee of the Physicians' Health Study Research Group. Final Report on the aspirin component of the ongoing physicians' health study. N Engl J Med 1989;321:129–35.
35. Tofler GH, Brezinski DA, Schafer AI, et al. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. N Engl J Med 1987;316:1514–8.
36. Muller JE, Tofler GH, Stone PH. Circadian variation and triggers of onset of acute cardiovascular disease. Circulation 1989;79:733–43.
37. Ridker PM, Manson JE, Buring JE, Muller JE, Hennekens CH. Circadian variation of acute myocardial infarction and the effect of low-dose aspirin in a randomized trial of physicians. Circulation 1990;82:897–902.
38. Peto R, Gray R, Collins R, et al. Randomised trial of prophylactic daily aspirin in British male doctors. Br Med J 1988;296:313–6.
39. Patrono C. Aspirin as an antiplatelet drug. N Engl J Med 1994;330:1287–94.
40. Hennekens CH, Dyken ML, Fuster V. Aspirin as a therapeutic agent in cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 1997;96:2751–3.
41. Shaeffer AI. Antiplatelet therapy. Am J Med 1996;101:199–209.
42. Folts JD, Rowe GG. Epinephrine potentiation of in vivo stimuli reverses aspirin inhibition of platelet thrombus formation in stenosed canine coronary arteries. Thrombosis Res 1988;50:507–16.
43. Folts JD, Gering SA, Laibly SW, Bertha BG, Bonebrake FC, Keller JW. Effects of cigarette smoke and nicotine on platelets and experimental coronary artery thrombosis. In: Diana JN, ed. Tobacco Smoking and Atherosclerosis. Pathogenesis and Cellular Mechanisms. New York: Plenum Press 1990;273: 339–58.
44. Roux SP, Sakariassen KS, Turitto VT, Baumgartner HR. Effect of aspirin and epinephrine on experimentally induced thrombogenesis in dogs. Arterioscl Thromb 1991;11:1182–91.
45. Lauri D, Cerletti C, deGaetano G. Amplification of primary response of human platelets to platelet-activating factor: Aspirin-sensitive and aspirin-insensitive pathways. J Lab Clin Med 1985;105:653–8.
46. Mittleman MA, Maclure M, Sherwood JB, et al. for the Determinants of Myocardial Infarction Onset Study Investigators. Triggering of acute myocardial infarction onset by episodes of anger. Circulation 1995;92:1720–5.
47. Moake JL, Turner NA, Stathopoulos NA, Nolasco L, Hellums JD. Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by endogenous large or unusually large vWF multimers, requires adenosine diphosphate and is resistant to aspirin. Blood 1988;71:1366–74.
48. Maalej N, Folts JD. Increased shear stress overcomes the antithrombotic platelet inhibitory effect of aspirin in stenosed dog coronary arteries. Circulation 1996;93:1201–5.
49. Wagner CT, Kroll MH, Chow TW, Hellums JD, Schafer AI. Epinephrine and shear stress synergistically induce platelet

PAGE 64/79 * RCVD AT 2/9/2005 5:54:17 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID: * DURATION (mm-ss):52-18

JACC Vol. 33, No. 2, 1999
February 1999:295-303

aggregation via a mechanism that partially bypasses VWF-GP Ib interactions. Biorheology 1996;33:209–29.
50. Mori TA, Vandongen R, Douglas AJ, McCulloch RK, Burke V. Differential effect of aspirin on platelet aggregation in IDDM. Diabetes 1992;41:261–6.
51. D'Souza D, Wu KK, Hellums JD, Phillips MD. Platelet activation and arterial thrombosis. Lancet 1994;344:991–5.
52. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non-responder. A pilot study including 180 post-stroke patients. Thrombosis Res 1993;71:397–403.
53. Lam JYT, Chesebro JH, Steele PM, et al. Antithrombotic therapy for deep arterial injury by angioplasty. Circulation 1991;84:814–20.
54. Meyer BJ, Badimon JJ, Mailhac A, et al. Inhibition of growth of thrombus on fresh mural thrombus. Targeting optimal therapy. Circulation 1994;90:2432–8.
55. Becker RC, Bovill EG, Corrao JM, et al. Platelet activation determined by flow cytometry persists despite antithrombotic therapy in patients with unstable angina and non-Q-wave myocardial infarction. J Thromb Thrombolysis 1994;1:95–100.
56. Smith JB. Prostaglandins in platelet aggregation. In: Bloom AL, Thomas DP, eds. Hemostasis and Thrombosis. New York: Churchill Livingstone, 1987:78–89.
57. Flores-Rank P, Raasch RH. Ticlopidine and antiplatelet therapy. Ann Pharmacother 1993;27:1090–8.
58. Splawinska B, Kuzniar J, Malinga K, Mazurek AP, Splawinski J. The efficacy and potency of antiplatelet activity of ticlopidine is increased by aspirin. Int J Clin Pharmacol Therapeut 1996;34:352–6.
59. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events. 1996;348:1329–39.
60. Savi P, Heilmann E, Nurden P. Clopidogrel: an antithrombotic drug acting on the ADP-dependent activation pathway of human platelets. Clin Appl Thromb Hemost 1996;2:35–42.
61. Lefkovits J, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. N Engl J Med 1995;332:1553–9.
62. Schafer AI, Alexander RW, Handin RI. Inhibition of platelet function by organic nitrate vasodilators. Blood 1980;55:649–54.
63. Folts JD, Stamler J, Loscalzo J. Intravenous nitroglycerin infusion inhibits cyclic blood flow responses caused by periodic platelet thrombus formation in stenosed dog coronary arteries. Circulation 1991;83:2122–7.
64. Rovin JD, Stamler JS, Loscalzo J, Folts JD. Sodium nitroprusside, an endothelium-derived relaxing factor congener, increases platelet cyclic GMP levels and inhibits epinephrine-exacerbated in vivo platelet thrombus formation in stenosed canine coronary arteries. J Cardiovasc Pharmacol 1993;22:626–31.
65. Golino P, Cappelli-Bigazzi M, Ambrosio G, et al. Endothelium-derived relaxing factor modulates platelet aggregation in an in vivo model of recurrent platelet activation. Circ Res 1992;71:1447–56.
66. Chirkov YY, Naujalis JI, Sage RE, Horowitz JD. Antiplatelet effects of nitroglycerin in healthy subjects and in patients with stable angina pectoris. J Cardiovasc Pharmaco 1993;21:384–9.
67. L-Lacoste L, Theroux P, Lidon RM, Colucci R, Lam JYT. The antithrombotic properties of transdermal nitroglycerin in stable angina pectoris. Am J Cardiol 1994;73:1058–62.
68. Andrews R, May JA, Vichers J, Hepinstall S. Inhibition of platelet aggregation by transdermal glycerol trinitrate. Br Heart J 1994;75:575–9.
69. Loscalzo J. Antiplatelet and antithrombotic effects of organic nitrates. Am J Cardiol 1992;70(Suppl B):18–22.
70. Amano M, Takahashi M, Kosaka T, Kinoshita M. Differential inhibition of platelet aggregation and calcium mobilization by nitroglycerin and stabilized nitric oxide. J Cardiovasc Pharmacol 1994;24:860–6.
71. Stamler JS, Loscalzo J. The antiplatelet effects of organic nitrates and related nitroso compounds in vitro and in vivo and their relevance to cardiovascular disorders. J Am Coll Cardiol 1991;18:1529–36.
72. Yusuf S, MacMahon S, Collins R, Peto R. Effect of intravenous nitrates on mortality in acute myocardial infarction: an overview of the randomized trials. Lancet 1988;2:1088–92.
73. GISSI-3: effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. Lancet 1994;343:1115–22.
74. ISIS-4 (Fourth International Study of Infarct Survival) Collaborative Group. ISIS-4: A randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58,050 patients with suspected acute myocardial infarction. Lancet 1995;345:669–82.
75. Langford EJ, Brown AS, Wainwright RJ, et al. Inhibition of platelet activity by S-nitrosoglutathione during coronary angioplasty. Lancet 1994;344:1458–60.
76. Bonebrake FC, Bertha B, Folts JD, Rao GH. Verapamil combined with aspirin for inhibiting epinephrine-stimulated platelet thrombus formation in stenosed canine coronary arteries. Coron Art Dis 1991;2:487–92.
77. L-Lacoste L, Lam JYT, Hung J, Waters D. Oral verapamil inhibits platelet thrombus formation in humans. Circulation 1994;89:630–4.
78. Folts JD. Inhibition of platelet activity in vivo by amlodipine alone and combined with aspirin. Int J Cardiol 1997;62(Suppl):111–7.
79. Held PH, Yusuf S, Furberg CD. Calcium channel blockers in acute myocardial infarction and unstable angina: an overview. Br Med J 1989;299:1187–92.
80. Stein B, Fuster V. Clinical pharmacology of platelet inhibitors. In: Fuster V, Verstraete M, eds. Thrombosis in Cardiovascular Disorders. Philadelphia: WB Saunders, 1992;107.
81. Folts JD, Rowe GG. Dipyridamole alone or with low-dose aspirin neither inhibits thrombus formation in stenosed canine coronary arteries nor protects against renewal of thrombus formation by epinephrine. J Vasc Med Biol 1989;1:255–61.
82. Coller BS, Smith SR, Scudder LE, Jordan R, Folts JD. Abolition of in vivo platelet thrombus formation in primates with monoclonal antibodies to the platelet GPIIb/IIIa receptor: correlation with bleeding time, platelet aggregation and blockade of GPIIb/IIIa receptors. Circulation 1989;80:1766–74.
83. Epic Investigators. Use of a monoclonal antibody directed against the platelet glycoprotein IIb-IIIa receptor in high risk coronary angioplasty. N Engl J Med 1994;330:956–61.
84. Topol EJ, Califf RM, Weisman HF, et al. Randomized trial of coronary intervention with antibody against the platelet IIb-IIIa integren for reduction of clinical restenosis: results at 6 months. Lancet 1994;343:881–6.
85. Simoons ML, de Boer MJ, van den Brand MJBM, et al. Randomized trial of a GPIIb/IIIa platelet receptor blocker in refractory unstable angina. Circulation 1994;89:596–603.
86. Tcheng JE. Perspectives on the future of platelet glycoprotein IIb/IIIa blockade therapy. Texas Heart Inst J 1998;25:49–56.
87. Ferguson JJ, Lau TK. New antiplatelet agents for acute coronary syndromes. Am Heart J 1998;135(Suppl):194–200.